REMARKS

The present application is directed to a method of causing expression of a desired heterologous protein in gastrointestinal mucosal cells of a mammal. The application also includes a method of inducing a serum or mucosal antibody response against *Yersinia pestis*. The recombinant gut-colonizing bacteria are useful for protecting humans against potential biological warfare agents such as plague.

Following entry of this amendment Claims 1, 23, 26-27, 29-31 and 33-35 will be pending. Claims 1, 26, 30, 31 and 33 are amended. Claims 2-22, 24-25, 28 and 32 are cancelled without prejudice. No new matter is added and support for the amendments can be found throughout the specification.

Allowable Subject Matter

The Examiner and applicants' representative, Dr. Zara Doddridge, conducted telephonic interviews on December 26, 2007 and January 2, 2008. The pending claims were discussed and claim amendments that would place Claims 33-35 in condition for allowance were finalized. Accordingly, applicants have amended Claims 33-35 herein to reflect the allowable subject matter discussed. Applicants kindly request allowance of amended Claims 33-35.

Examiner Interview

Applicants' representative, Dr. Zara Doddridge, conducted a telephonic interview with Examiner Devi on May 12, 2008, to request clarification of information provided in the Advisory Action. The Examiner had not indicated whether the amendment filed April 14, 2008, in response to the Final Office Action would be entered. (Neither part (a) nor part (b) of PTOL-303 was checked.) During the telephone interview, the Examiner stated that the amendments filed April 14, 2008, would **not** be entered for the purposes of appeal because the amendments raise new issues that would require further consideration and/or search. Accordingly, applicants timely file this Request for Continued Examination.

Claim rejections under 35 U.S.C. § 112, first paragraph

In the Final Office Action mailed January 14, 2008, the Examiner rejected Claim 1 and dependent Claims 23 and 25-32 under 35 U.S.C. § 112, first paragraph, as containing new matter. Applicants respectfully submit that the amendments to the claims overcome the rejection.

Claims 25 and 32 were previously cancelled in an earlier Amendment and Response to Office Action. Accordingly, applicants have interpreted the instant rejection to cover Claims 1, 23 and 26-31.

Claim 1 is amended herein to clarify that the gastrointestinal mucosal cells are present in the mammal to which the recombinant gut-colonizing bacterium is administered. In addition, Claim 1 relates to a method of causing expression of a desired **heterologous** protein (i.e. not native to the bacterium) in the mucosal cells of the mammal, wherein the recombinant gut-colonizing bacterium is suitably attenuated such that the mammal does not experience significant harmful effects as a result of infection by the recombinant gut-colonizing bacterium.

Support for the above amendments can be found in the specification. In particular, applicants respectfully direct the Examiner to the "Abstract" of the instant application that recites "*a method of enhancing expression of a desired protein at mucosal effector sites...*" and "*...causing expression in mucosal cells*".

Furthermore, page 4, lines 1-4 of the instant application recites that certain promoters, such as P_{phoP}, and P_{pagC}, and P_{ompC}, corresponding to SEQ ID NOs: 2-4, respectively, "*can be used to advantageously in such systems to drive high levels of expression of heterologous proteins, in particular in mucosal cells*". Applicants also submit that page 6, lines 8-14, discloses that "*the finding that mucosal antibody **in the gut** was induced only after immunisation with recombinant Salmonella expressing F1-antigen from the phoP or pagC gene promoters suggests that these promoters **directed high-level expression** of F1-antigen **within GALT***" (Gut-Associated Lymphoid Tissue)(emphasis added). Additionally, applicants respectfully submit Example 5, found on page 18, lines 14-20 discloses **intragastric** (i.g.) dosing of a recombinant gut-colonizing bacterium to a mammal (this example was carried out in mice).

Applicants respectfully direct the Examiner to page 6, lines 23-26, of the instant application wherein applicants disclose that the "*recombinant gut-colonizing microorganisms of*

the invention are suitably attenuated so that the host does not experience significant harmful effects as a result of infection by the microorganism". Further explanation as to why page 6, lines 23-26 provides support for "gastrointestinal" is given below (see section on T and B cells). In brief, for physiological reasons, the heterologous protein will not have been delivered to mucosal cells other than gastrointestinal ones. In contrast to what is stated on page 4 of the Final Office Action, applicants claim a **suitably attenuated** recombinant gut-colonizing bacterium, and not a **generic** recombinant gut-colonizing bacteria. Applicants respectfully submit that basis for "*suitably attenuated*" recombinant gut-colonizing microorganisms can be found on page 6, lines 23-26 of the specification.

Applicants note that the Examiner raised on page 4 of the Office Action "*that claim 1 as originally filed neither included oral administration step nor expression of a heterologous protein in generic mucosal cells*". As discussed above, there are several examples within the specification that support the claim limitations as recited in amended Claim 1. Additionally, Claim 20 as filed was directed to oral administration of a vaccine comprising the recombinant gut-colonizing microorganism as also described on page 8, lines 21-23 of the instant application.

Claim 26 is amended herein to depend from Claim 1 and to correct a typographical error. Claim 31 is also amended herein to correct a typographical error.

For at least the foregoing, applicants respectfully request withdrawal of the new matter rejection under 35 U.S.C. §112, first paragraph.

In the January 14, 2008, Final Office Action, the Examiner rejected Claims 1, 23 and 25-32 under 35 U.S.C. §112, first paragraph, for lack of enablement. Applicants respectfully submit that the amendments to the claims overcome the rejection.

As discussed above, Claim 1 is directed to a method of expressing a desired heterologous protein in gastrointestinal mucosal cells of a mammal by placing a nucleotide sequence encoding the heterologous protein under the control of a promoter consisting of a nucleotide sequence of SEQ ID NO: 2, the promoter being operatively interconnected to the nucleotide sequence encoding the desired heterologous protein, in a recombinant gut-colonizing

bacterium, wherein the recombinant gut-colonizing bacterium is suitably attenuated so that the mammal does not experience significant harmful effects as a result of infection by the recombinant gut-colonizing bacterium, and administering the recombinant gut-colonizing bacterium orally to the mammal.

Applicants respectfully submit that the desired heterologous protein is **not** expressed in nasal or vaginal cells (generic mucosal cells) as suggested by the Examiner because it is **T and B cells** primed against the desired heterologous protein that migrate to other mucosal sites rather than migration of the **heterologous protein itself or the recombinant gut-colonizing bacterium**. Applicants submit that recombinant gut-colonizing bacteria are administered orally to a mammal and that the recombinant gut-colonizing bacteria are engulfed into Peyer's patches (containing the gastrointestinal mucosal cells) where B and T cells are primed against the bacteria **resulting in migration of the T and B cells to other mucosal surfaces**. In support of the above remarks, applicants submit herewith an extract from a textbook published in 1994, entitled "***Bacterial Pathogenesis: A Molecular Approach***" by Salyers and Whitt (Exhibit A). Applicants respectfully direct the Examiner to page 9, right-hand column, first full paragraph beginning "After MALT B and T cells..." through to, same paragraph, "the **secondary migration** of MALT **T and B** cells to ***other*** mucosal surfaces spreads immune protection to these other areas" (emphasis added) that describes the migration of B and T cells from the original mucosal surface site where they were primed (gastrointestinal mucosal cells) to other (i.e. non-gastrointestinal) mucosal surfaces in response to an antigen and not the migration of a heterologous protein or a recombinant gut-colonizing bacterium to a non-gastrointestinal mucosal surface. Applicants respectfully submit that Claim 1 as previously amended was already limited to expression of heterologous proteins in gastrointestinal mucosal cells, and Exhibit A provides a clear explanation of a host's response upon exposure to an antigen. However, Claim 1 has been further amended to include "gastrointestinal" in order to make this more explicit.

The Examiner also maintains that the claimed method would require undue experimentation by one of ordinary skill in the art to apply the method to other heterologous

proteins or generic mucosal cells owing to a large number of possible variables in the choice of heterologous protein, and choice of mucosal cells, etc.

To the contrary, applicants respectfully submit that one of ordinary skill in the art, contemplating using the claimed method, is not going to decide which heterologous protein, from all known heterologous proteins, should be selected to use in the present expression system. Instead, one of ordinary skill in the art is **already** going to have a heterologous protein in mind that he/she wishes to express using the present expression system.

At its simplest, one of ordinary skill in the art need only substitute the polynucleotide sequence encoding for example, an F1 antigen of *Yersinia pestis* as shown in Example 2, for a polynucleotide sequence encoding their desired heterologous protein. Molecular biology techniques such as PCR and restriction digests that would enable one of ordinary skill in the art to perform such a task are well-known. Applicants respectfully submit that the claimed method does not therefore place an undue burden of experimentation on one of ordinary skill in the art.

Similarly, applicants respectfully submit that it is not an undue burden to switch from one suitably attenuated recombinant gut-colonizing bacterium to another. Bacteria have long been used as expression systems in molecular biology and one of ordinary skill in the art looking to express a desired heterologous protein would know how to incorporate a polynucleotide sequence encoding the desired heterologous protein into an appropriate bacterium. Applicants respectfully assert that substitution of one suitably attenuated recombinant gut-colonizing bacterium for another is considered routine in the art. Indeed, applicants provide a plasmid construction for the preparation of a recombinant gut-colonizing bacterium in Figure 1 of the instant application. Thus, the number of variables that one of ordinary skill in the art needs to consider in order to carry out the claimed invention is actually very few.

Additionally, the number of variables is further reduced because, as already discussed, the claimed method is limited to expression of heterologous proteins in **gastrointestinal** mucosal cells and not mucosal cells in general.

Furthermore, applicants respectfully submit that the method as disclosed in the instant Examples and Figures of the specification allows one of ordinary skill in the art to make

and use the claimed method. Applicants kindly direct the Examiner to the Figures on page 12, lines 9-35, of the present application. Specifically, Figure 5 shows comparative antibody responses against a desired heterologous protein using various promoters (including the *phoP* promoter), in the serum (i.e. circulating), and in the gut and lung. Figure 7a shows comparative mucosal antibody levels (IgA) against a desired heterologous protein in Peyer's patches containing the gastrointestinal mucosal cells when using different promoters (including the *phoP* promoter).

Additionally, applicants respectfully submit that significant guidance in the form of working Examples is provided on pages 13-21 of the specification. In particular, applicants disclose preparation of bacterial strains, the *in vitro* stability of different plasmids encoding a F1-antigen in *S. typhimurium*. The stability of the different plasmids was also evaluated *in vivo* (see Example 3). Serum and mucosal antibody responses to the F1-antigen are presented (see Examples 4 and 5). Peyer's patches were also removed to determine the presence of F1-antigen and *Salmonella* specific IgA producing cells in the gut (Example 6). Accordingly, applicants respectfully submit that the instant application provides a considerable amount of guidance and direction and that the amended claims as provided herein are supported and enabled.

Accordingly, applicants respectfully submit that the specification as filed is sufficient to enable one of ordinary skill in the art to make or use the invention commensurate with the pending claims.

For at least the foregoing reasons, applicants respectfully request withdrawal of the enablement rejections under 35 U.S.C. §112, first paragraph.

Claim rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected Claims 1, 26-31 and 33-35 under 35 U.S.C. § 112, second paragraph, as indefinite. Applicants respectfully submit that amendments to the claims overcome the rejection.

Claim 1 is amended herein to correct antecedent basis for the limitation "the mucosal cells of the mammal." Applicants respectfully submit that amended Claim 1 is definite and kindly request withdrawal of the rejection.

Claim 28 is cancelled herein. Applicants respectfully submit that the rejection of Claim 28 is moot and kindly request withdrawal of the rejection.

Claim 33 is amended herein to clarify that the "induced serum or mucosal antibody response" is directed to a mammal. Claim 33 is also amended herein to clarify the "F1-antigen of *Yersinia pestis",* and "the nucleotide sequence" as suggested by the Examiner. Applicants respectfully submit that amended Claim 33 is definite and kindly request withdrawal of the rejection.

Accordingly, applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

CONCLUSION

The foregoing is submitted as a full and complete Response to the Final Office Action mailed on January 14, 2008. For at least the reasons given above, applicants respectfully submit that the pending claims are described in the specification, enabled and definite. Accordingly, applicants submit that the claims in the present application are in condition for allowance, and such action is courteously solicited.

If the Examiner believes there are other issues that can be resolved by telephone interview, or that there are any informalities remaining in the application that may be corrected by Examiner's Amendment, a telephone call to the undersigned agent at (404)815-6473 is respectfully solicited.

No additional fees are believed due; however the Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account number 11-0855.

Respectfully submitted,

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